

Phylogeny for the Tribe Thophini (Cicadoidea: Cicadidae) with the Description of a New Subspecies of *Thopha sessiliba* Distant from Western Australia

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ABSTRACT. A molecular phylogeny for the cicada tribe Thophini (*Thopha* + *Arunta*) is provided together with a cladistic analysis based on morphological data. A new subspecies, *Thopha sessiliba clamoris*, is described from the eastern fringe of the Pilbara region of Western Australia, based on molecular, morphological, and behavioral evidence. All described species of *Thopha* are figured and a revised key to the five species and two subspecies provided. A discussion on the biogeography of the genus is also included.

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The two genera of the tribe Thophini, *Thopha* Amyot & Serville and *Arunta* Distant, are recognized by greatly swollen timbal covers (Moulds, 2005, 2012). *Thopha* species inhabit *Eucalyptus* trees while *Arunta* species are found in mangroves or trees growing on coastal sand dunes, primarily *Banksia integrifolia* and *Casuarina* species (Moulds, 1990).

In a recent paper (Moulds, 2008), a colour form of *Thopha sessiliba* Distant was recorded from Western Australia, west of the Great Sandy Desert. This form showed distinctive bold markings on the head and thorax, especially evident on the pronotum. The differences in markings were so distinct that it was believed that the western form represented another species, but this argument was abandoned when differentiating molecular and other evidence was found to

be absent. Here we present molecular evidence showing that western *T. sessiliba* are a separate evolutionary lineage but insufficiently distinguished to warrant species status although, in conjunction with their striking colour difference and geographic isolation, worthy of subspecific status. A formal description follows. We also provide an analysis of the song and compare songs of allied species.

Molecular and morphological data have also been used to investigate phylogenetic relationships between all seven species of the tribe Thophini, viz. the five species of *Thopha* and the two species of *Arunta*. Molecular results are compared with morphological results and song measurements, and a phylogeny of the combined data is presented.

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Terminology for morphological features follows Moulds (2005). The following abbreviations have been used for collections housing specimens: *AE*, collection of A. Ewart; *AM*, Australian Museum, Sydney; *AS*, collection of Allen Sundholm; *PH*, collection of Paul Hutchinson; *MSM*, collection of M. S. Moulds; *WAM*, Western Australian Museum, Perth.

Thopha sessiliba clamoris subsp. n.

Figures 1–5

Type material. Holotype ♂, c.221 km S of Newman, site code WA.GRN, 25°06.355'S 119°22.369'E, 545 m, 14 Feb. 2006, Hill, Marshall, Moulds (WAM). —Paratypes (24 ♂♂, 8 ♀♀): 1♂ (molecular voucher 06.AU.WA.KUM.10), c.167 km S of Newman, 24°42.759'S 119°36.513'E, 607 m, 13 Feb. 2006, Hill, Marshall, Moulds; 1♂, 1♀, 45 km S of Gascoyne River, 146 km NNE of Meekatharra, site code WA.GRS, 25°35.579'S 119°14.173'E, 545m, 18 Feb. 2009, K. Hill and D. Marshall (AMS). 1♂, 3♀♀, Landor Meekatharra Road, 26°28'04.00"S, 118°06'19.70"E, 41 km W of Meekatharra, 19.i.2014, Allen M. Sundholm, T.M.S. Hanlon (AS). 1♂, 210 km S of Newman, site code WA.KSC, 25°01.126'S 119°24.560'E, 559 m, 13 Feb. 2006, Hill, Marshall, Moulds; 1♂, c.221 km S of Newman, site code WA.GRN, 25°06.355'S 119°22.369'E, 545 m, 14 Feb. 2006, Hill, Marshall, Moulds; 1♂ (genitalia preparation THOP23, molecular voucher 06.AU.WA.GRS.04), 1♀, 146 km NNE of Meekatharra, 25°35.579'S 119°14.173'E, 545m, 14 Feb. 2006, Hill, Marshall, Moulds; 1♂, 1♀, 55 km NNE of Meekatharra, site code WA.YAL, 26°08.225'S 118°41.721'E, 478 m, 18 Feb. 2009, K. Hill and D. Marshall (MSM). 1♂, 192 km N of Meekatharra, 7 Mar. 2006, P. Hutchinson; 7♂♂, 146 km N of Meekatharra, 7 Mar. 2006, P. Hutchinson; 2♂♂, 57 km N of Meekatharra, 6 Mar. 2006, P. Hutchinson (PH). 1♂, 1♀, c.221 km S of Newman, site code WA.GRN, 25°06.355'S 119°22.369'E, 545 m, 14 Feb. 2006, Hill, Marshall, Moulds; 1♂, 146 km NNE of Meekatharra, site code WA.GRS, 25°35.579'S 119°14.173'E, 545m, 14 Feb. 2006, Hill, Marshall, Moulds; 2♂♂, 1♀, 45 km S of Gascoyne River, 146 km NNE of Meekatharra, 25°35.579'S 119°14.173'E, 545m, 18 Feb. 2009, K. Hill and D. Marshall; 1♂, 55 km NNE of Meekatharra, 26°08.225'S 118°41.721'E, 478 m, 14 Feb. 2006, Hill, Marshall, Moulds; 2♂♂, same locality, 18 Feb. 2009, K. Hill and D. Marshall; (WAM). All Western Australia.

Etymology. The subspecific epithet is derived from the Latin *clamor*, meaning loud call.

Description

Male (Figs 1a–c, 5). *Head* muddy orange; vertex with a reddish brown fascia between eyes terminating just short of each eye and ill-defined around ocelli; much of ventral surface reddish brown. Postclypeus mostly reddish brown, pale muddy orange dorsally. Anteclypeus reddish brown and a little paler than postclypeus; narrowly edged black; often reddish brown along part of or entire midline, with a small median expansion. Lorum reddish brown with muddy yellow margin. Rostrum with mentum and labium muddy orange; labium dark reddish brown along groove, apical portion partly black; reaching almost to apices of hind coxae. Antennae with scape and pedicel reddish brown, the latter very dark to nearly black apically; flagellum black or nearly so.

Thorax. Pronotum muddy orange, pronotal collar tending slightly paler; lateral margin of pronotal collar partly narrowly edged black or dark reddish brown; midline boldly marked by a dark reddish brown fascia (often with a pale narrow interior) that does not quite reach the anterior pronotal margin or the postclypeus, this fascia expands abruptly at its distal end and gradually expands towards its anterior end; lateral and submedian sigilla dark reddish brown, sometimes nearly black; midline usually with a dark reddish brown fascia, narrowly tapering and pointed anteriorly and reaching submedian sigilla, the posterior end reaching to cruciform

elevation and sometimes expanded to merge with similarly coloured scutal depressions; white pubescent band along lateral margin adjacent to wing groove, tapering posteriorly. Metanotum muddy orange. Thorax on underside muddy orange to dark reddish brown; extensively covered by fine white pubescence.

Wings hyaline and without infuscations. Fore wing venation muddy orange; subcosta jet black along basal cell, jet black along 2A+3A in the vicinity of basal membrane. Basal cell opaque muddy orange but usually with a small window adjacent to CuA. Basal membrane bright orange. Hind wing venation yellow or orange; plaga following CuP+2A and 3A orange.

Legs dark reddish brown tending partly muddy orange. Coxae usually with dense white pubescence on outer face; fore coxae with muddy pale orange stripe for full length on outer face; mid and hind coxae usually tending muddy orange. Fore femora tending muddy orange on outer face, dark reddish brown on inner face. Pretarsal claws black with dark brown suffusion at base.

Opercula with their apices clearly not meeting but nevertheless close; muddy yellow but usually covered by pure white pubescence.

Abdomen. Tergites very dark reddish brown to almost black; tergite 2 with a large central patch of fine white pubescent “dusting”, epipleurites 3–7 with similar white pubescence extending onto adjacent sternites; tergite 8 usually completely covered by white pubescence. Sternites very dark reddish brown to almost black; sternites I and II with some white pubescence, usually most distinct on distolateral extremities of sternite I and along anterior margin of II.

Timbals. Timbal covers dark reddish brown, usually with dense, pure white pubescence below adjacent to rim. Timbals similar to those of other *Thopha* species; four long ribs, the first against the margin of the timbal plate and second very broad and joined dorsally with third; one short, very broad rib between second and third long ribs.

Genitalia (Figs 1b–c). Distal shoulders of pygofer weakly developed with distal portion bluntly pointed and turned backwards. Pygofer basal lobes not well developed, completely or substantially hidden in lateral view. Uncus deeply divided into a pair of long, gently-tapering, tooth-like lobes, in dorsal view diverging, in lateral view angled downwards at about 45°; ventral tooth completely fused with uncus lobe except towards apex which is short, bluntly rounded, shorter than uncus lobe.

Female. Similar to male. Tergite 8 covered by pure white “dusting” of microscopic pubescence. Abdominal segment 9 very dark reddish brown to black; much of lateral surface and adjoining distal half or so of dorsal midline covered by white pubescent “dusting”.

Measurements. Range and mean (in mm) for 10 males and 6 females; includes smallest and largest of available specimens. *Length of body (including ovipositor)*: male 42.8–49.1 (46.4); female 44.4–48.4 (47.0). *Length of fore wing*: male 55.0–59.8 (57.6); female 55.0–59.5 (58.0). *Width of head (including eyes)*: male 20.0–21.8 (21.1); female 20.1–22.0 (21.6). *Width of pronotum (across lateral angles)*: male 17.7–19.6 (18.7); female 17.2–19.4 (18.9).

Distinguishing morphological features. *Thopha sessiliba clamoris* (Fig. 5) can be distinguished from *T. sessiliba sessiliba* (Fig. 5) and all other species of *Thopha* by the lack

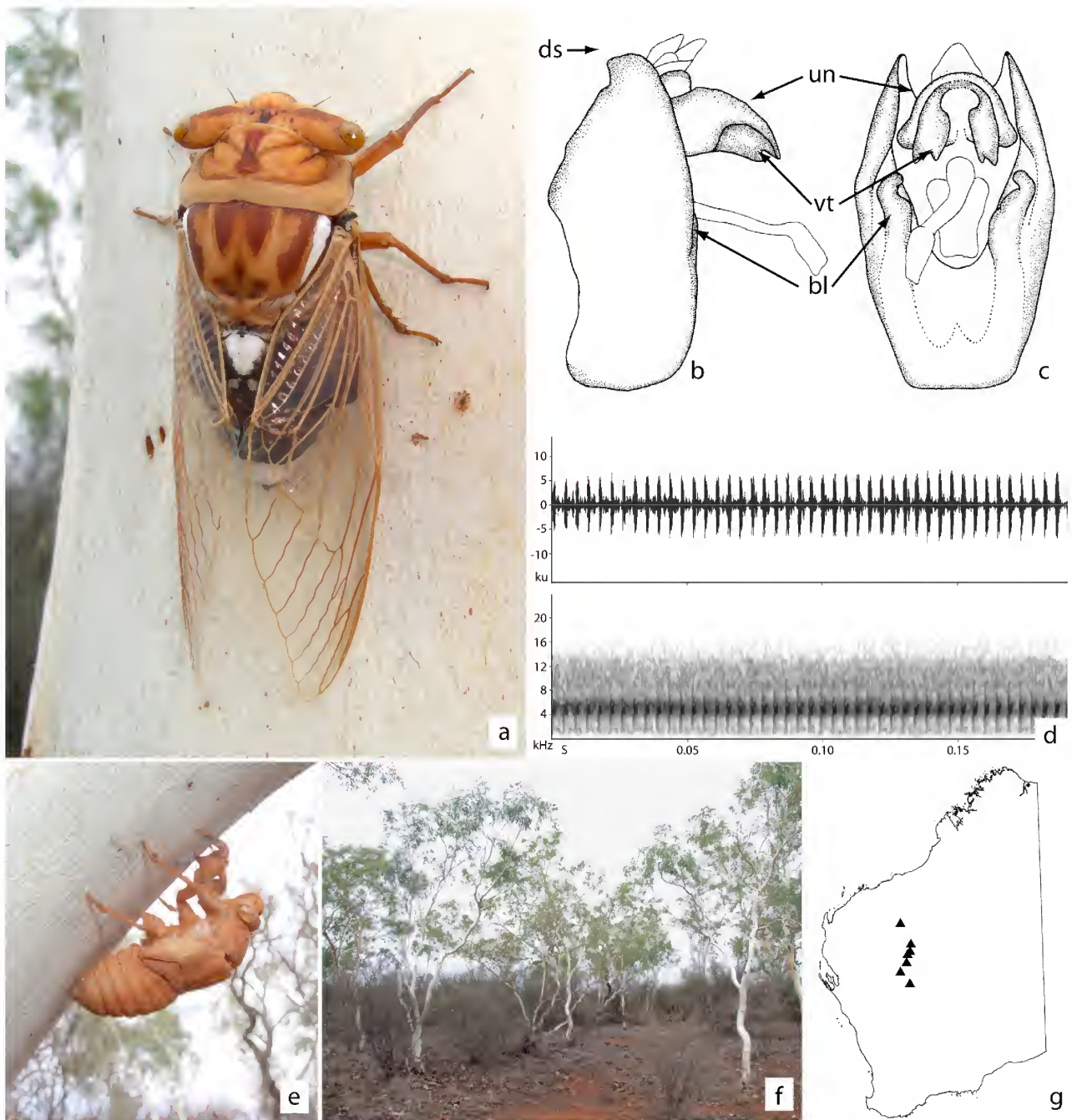


Figure 1. *Thopha sessiliba clamoris* subsp. n. (a) male live on *Eucalyptus* sp.; (b, c) male genitalia, lateral and ventral views respectively, specimen from 146 km NNE of Meekatharra, Western Australia, *bl* = basal lobe, *ds* = distal shoulder, *un* = uncus, *vt* = ventral tooth; (d) partial song phrase from male specimen 09.AU.WA.GRS.01 (see Table 2), oscillogram shown above sonogram; (e) nymph shell on *Eucalyptus* sp.; (f) *Eucalyptus* sp. habitat at 55 km NNE of Meekatharra, Western Australia; (g) distribution map showing known locations in Western Australia.

of bold, jet-black markings on head and thorax, the presence of a bold, dark reddish brown central fascia on the pronotum, and the similar colouration on the pronotum and pronotal collar. Further, the dark reddish brown fascia across the head between the eyes terminates just before the eyes, whereas this fascia nearly always meets the eyes in *T. s. sessiliba*. Also, in *T. s. clamoris* there is always obvious dark reddish brown pigmentation surrounding the scutal depressions; in *nearly all* specimens of *T. s. sessiliba* the scutal depressions are clearly marked but the dark pigmentation does not extend beyond the depressions.

Distribution and habitat (Figs 1f–g, 2). Known only from the eastern fringe of the Pilbara region, Western Australia. Specimens have been taken at several locations along the Great Northern Highway between 167 km south of Newman and 55 km NNE of Meekatharra. The region is semi arid receiving approximately 200–300 mm average annual rainfall that comes mainly as summer thunderstorms and cyclones.

Adults frequent river gums, *Eucalyptus camaldulensis*, preferring the main trunk and limbs. For the most part these trees grow along sandy or stony stream banks that

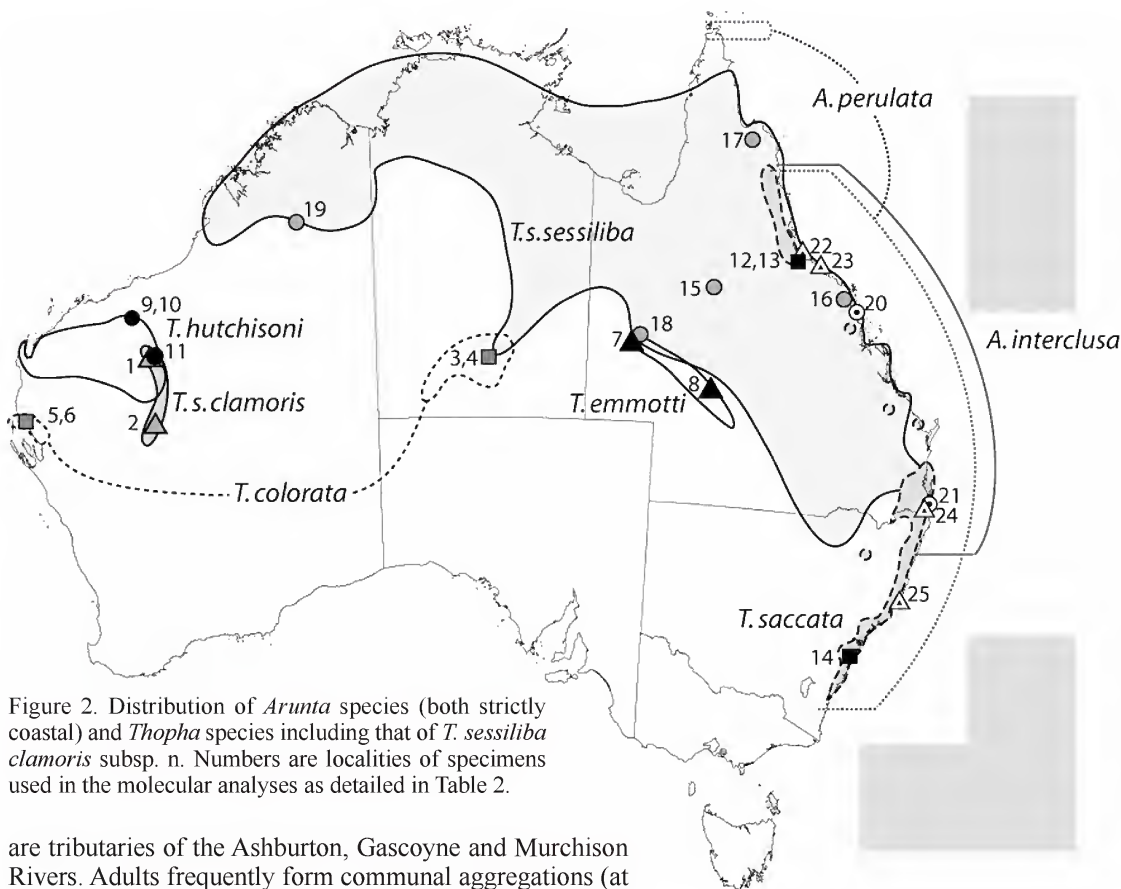


Figure 2. Distribution of *Arunta* species (both strictly coastal) and *Thopha* species including that of *T. sessiliba clamoris* subsp. n. Numbers are localities of specimens used in the molecular analyses as detailed in Table 2.

are tributaries of the Ashburton, Gascoyne and Murchison Rivers. Adults frequently form communal aggregations (at the northern limit of its range sometimes occurring with *T. hutchinsoni*), with several individuals occupying a single tree or group of adjacent trees. The larger a tree, usually the greater the aggregation within it.

Adults emerge after heavy summer and early autumn rains and have been taken in mid-February and early March. Because of this dependence upon heavy rain for emergence, combined with the unreliable nature of the rainfall, the appearance of adults is probably erratic.

Singing occurs during the heat of the day and at dusk. The song is a loud, drone-like pulsating whine, very similar to that of the other *Thopha* species (Figs 1d, 3). See below for analysis of song and comparisons to *T. s. sessiliba* and *T. hutchinsoni*.

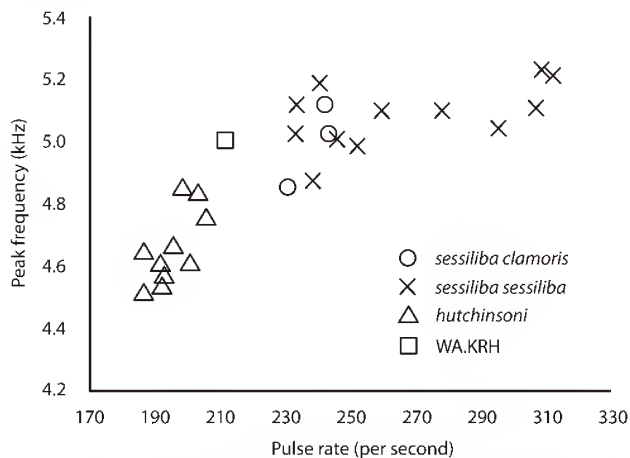


Figure 3. Plot of mean peak song frequency (dominant pitch) versus mean pulse rate (timal click rate) for *Thopha sessiliba clamoris* subsp. n., *T. sessiliba sessiliba* and *T. hutchinsoni*. The WA.KRH is a potential location of contact between *T. s. clamoris* and *T. hutchinsoni*.

Morphological cladistic analysis of Thophini

Cladistic analysis methods

Data for 16 characters (representing 19 states) were derived from adult morphology (thirteen characters), or colour pattern (three characters). The three multistate characters (characters 5, 8 and 12) were treated as unordered. Character polarity was determined using the outgroup species *Henicopsaltria rufivelum* Moulds, *Henicopsaltria* being previously identified as a close sister genus to *Thopha* (Moulds, 2005; C. Simon, pers. comm. from molecular data). Characters 4, and 12 are generic synapomorphies that separate *Thopha* species from its sister genus *Arunta* (identified as such in Moulds, 2005). Neither character weighting or successive weighting was employed. The number of scorable characters was limited by the close morphological similarity of *Thopha* species; two species, *T. hutchinsoni*, *T. sessiliba*, appear to show no morphological differentiation but are discernable by colour differences (Moulds, 2008) and molecular evidence (this paper). The matrix of taxa and assigned states is given in Table 1. The characters and character states employed were as follows:

- 1 *Width of head (including eyes)*: (0) about as wide as lateral margins of pronotal collar; (1) very much wider.
- 2 *Eyes*: (0) large, their maximum diameter equal to or greater than distance from eye to lateral ocellus; (1) small, their maximum diameter much less than distance from eye to lateral ocellus.
- 3 *Markings on head*: (0) mostly jet black; (1) none black.

Table 1. Data matrix for cladistic analysis.

Species	Characters					
	00000	00001	11111	1		
	12345	67890	12345	6		
<i>Henicopsaltria rufivelum</i> (outgroup)	00000	00000	00010	0		
<i>Arunta interclusa</i> (Walker)	01010	01011	20000	0		
<i>Arunta perulata</i> (Guérin-Méneville)	01010	01011	20000	0		
<i>Thopha colorata</i>	01001	01011	11110	1		
<i>Thopha emmotti</i>	11001	11011	11110	1		
<i>Thopha hutchinsoni</i>	11101	11111	11111	2		
<i>Thopha saccata</i>	01011	01011	10010	1		
<i>Thopha sessiliba sessiliba</i>	11101	21111	11111	2		
<i>Thopha sessiliba clamoris</i> subsp.n.	11101	21111	11111	2		

- 4 *Postclypeus* in dorsal view: (0) flattened, almost straight; (1) rounded, protruding.
- 5 *Pronotal collar*: (0) of moderate proportions, lateral margins narrow; (1) broad, lateral margins broad with straight outer margin.
- 6 *Median and lateral sigilla*: (0) black; (1) pale, ill-defined; (2) dark reddish brown.
- 7 *Opercula*: (0) well developed, elongate, overlapping; (1) short, much wider than long, not meeting.
- 8 *Fore wing costa and/or subcosta*: (0) brown to orange or greenish, never with black; (1) mostly jet black.
- 9 *Timbal covers*: (0) reaching backwards a little beyond second abdominal segment; (1) reaching backwards beyond third abdominal segment.
- 10 *Timbal covers*: (0) flat and nearly confluent with abdominal profile; (1) greatly swollen and sack-like.
- 11 *Timbal covers*: (0) lacking white wax; (1) partly covered by white waxy secretion; (2) entirely covered by white waxy secretion.
- 12 *Abdominal segment 8*: (0) pubescent without white wax or very minimal wax; (1) with substantial white wax, almost covering entire segment.
- 13 *Male tergites 2 and 3*: (0) tergite 2 about as wide as tergite 3; (1) tergite 2 much wider than tergite 3
- 14 *Pygofer dorsal beak*: (0) well developed, spine-like; (1) degenerate, missing or very small.
- 15 *Uncus*: (0) divided to about mid length; (1) deeply divided.
- 16 *Uncal ventral projection*: (0) large and rounded; (1) long, spine-like, sharply pointed; (2) small, rounded, tooth-like.

Data were analysed using the heuristic search parsimony algorithm implemented with PAUP* version 4.0b10 (TBR + RAS=10, MULPARS) (Swofford, 2001). Trees were prepared using Hennig86 and CLADOS version 1.2 (Nixon, 1992). Character numbers were adjusted to begin at “1”, rather than the “zero” default. Bootstrap values greater than 50% were generated through PAUP* using default parameters but 1,000 replications.

Key to species of *Thopha*

The key to *Thopha* species provided by Moulds (2001) and updated in Moulds (2008) is here presented in full with *T. sessiliba clamoris* incorporated (see also Fig. 5).

- 1 Head and thorax with bold, jet-black markings 4
 — Head and thorax lacking bold, jet-black markings 2
- 2 Mesonotum with thoracic sigilla ill-defined *T. hutchinsoni* Moulds
 — Mesonotum with thoracic sigilla boldly marked reddish brown 3
- 3 Pronotum with midline always boldly marked reddish brown
and colour of pronotal collar similar to remainder of pronotum *T. sessiliba clamoris* subsp. n.
 — Pronotum rarely with midline boldly marked reddish brown, *if so*
 then colour of pronotal collar obviously paler than remainder of
 pronotum *T. sessiliba sessiliba* Distant
- 4 Postclypeus orange or muddy yellow in dorsal view 5
 — Postclypeus black in dorsal and ventral view *T. saccata* (Fabricius)
- 5 Mesonotum with submedian sigilla marked partly or entirely jet
 black *T. colorata* Distant
 — Mesonotum with submedian sigilla not black but yellowish
 brown, similar in colour to mesonotum *T. emmotti* Moulds

Cladistic analysis—results

Analysis produced just one most parsimonious tree (Fig. 4a), with a length of 20 steps, a consistency index (CI) of 99, and a retention index (RI) of 97. The generic statuses of *Thopha* and *Arunta* are maintained as sister groups; *Thopha* supported by two non homoplasious synapomorphies but a weak bootstrap and *Arunta* by one non homoplasious and two homoplasious synapomorphies and a weak bootstrap. Within the *Thopha* clade, *T. saccata* is sister to the rest of the *Thopha* species well supported by two non homoplasious synapomorphies and bootstrap of 93%. Likewise, *T. colorata* and *T. emmotti* form a sister relationships with remaining species. The final clade, comprising *T. hutchinsoni*, *T. sessiliba sessiliba* and *T. sessiliba clamoris* subsp. n., is very well supported as a monophyletic group with four non homoplasious synapomorphies and a bootstrap of 99%. *Thopha s. clamoris* is sister to *T. s. sessiliba* but the relationship is only weakly supported while this pair is only weakly distinguished from *T. hutchinsoni* by one non homoplasious character.

Molecular phylogenetic analyses of Thophini

Molecular laboratory methods. *Thopha* and *Arunta* species and outgroup samples were collected from their known ranges throughout Australia. Tissue samples were preserved in 95% ethanol upon capture, or legs were broken from pinned specimens collected within the last 12 years. GPS coordinates were determined in the field or by extrapolating from high-definition maps if GPS was unavailable. DNA voucher specimens are deposited in the AM collection. As in the morphological analysis, *Henicopsaltria rufivelum* Moulds was chosen for the outgroup based on molecular studies of the family Cicadidae (Simon *et al.*, unpublished).

DNA was extracted from leg muscle using a *Qiagen DNeasy Blood and Tissue Kit* (Qiagen Inc., Valencia, California, USA) following the manufacturers' instructions but with a proteinase K digestion time of 18 h at 54°C. Standard polymerase chain reaction (PCR) methods were used to amplify two portions of DNA using an Ex Taq™ kit (Takara Bio Inc., Otsu, Shiga, Japan): approximately 1500 bp of the mitochondrial cytochrome oxidase subunit I (COI) gene using primers C1-J-1490 (Folmer *et al.*, 1994) and TL2-N-3014 (Simon *et al.*, 1994) and an annealing temperature of 45°C; and approximately 800 bp of the nuclear elongation factor 1 α (EF-1 α) gene using the primers EF1-PA-f650ambig (Lee & Hill, 2010) and EF-N-1419 (Sueur *et al.*, 2007) and an annealing temperature of 58°C. PCR products were visualised on a 1% agarose gel (BP1356-500 agarose Fisher Scientific, Pittsburg, PA) prior to clean up and purified using ExoSAP-IT™ (USB Corp., Cleveland, Ohio, USA). EF-1 α PCRs that amplified two bands were separated on a 1.5% agarose gel, cut out and purified using the Clontech Extract II™ kit (Clontech, Mountain View, California, USA). Purified PCR products were cycle sequenced in both directions using a standard cycle sequencing protocol (with BigDye™ 1.1, Applied Biosystems, Foster City, California, USA), and then sequenced on an ABI 3100 capillary sequencer with ABI Prism Sequencing Analysis 3.7 software (Applied Biosystems). Internal primers were also used within the large COI fragment to obtain clean sequence for the middle of the fragment; the primer(s) used were either C1-J-2195 (Simon *et al.*, 1994), or either of

three new primers constructed for Cicadinae by K.B.R. Hill: C1-J-2216 5'-GAAGTTTATATTTTTRATTTTACCTGG-3', C1-N-2390 5'-CCAGTTGGAAGTGAATAATTATAGTAG-3' or C1-N-2638 5'-TAYCARTGAAYAAATCTDCC-3'. Sequencher (Gene Codes Corporation, Ann Arbor, Michigan, USA) software was used to edit the raw sequence data, and the final alignment was performed by eye in *MacClade* 4.0 (Maddison & Maddison, 2000). Pairwise sequence divergences were calculated using Paup* v4.0b (Swofford, 2001).

Because some *Thopha* and *Arunta* individuals exhibited double peaks in the COI gene sequences that were attributed to nuclear copies of mtDNA (numts), the whole mitochondria of these individuals were also extracted and the mtDNA purified using the Wizard Plus SV Minipreps DNA Purification System kit (Promega, Madison, Wisconsin, USA) following the modifications of Beckman *et al.* (1993) and Sunnucks & Hales (1996). This protocol uses alkaline lysis to isolate the mitochondria in a crude separation from the nuclear DNA and has been used in previous studies to avoid numts (e.g., Ibaraguchi, 2006). PCR for the COI gene was then carried out on the purified mtDNA using the same protocol as above. PCR for the EF-1 α gene was also trialled on two of the purified mtDNA extractions to check for the presence of nuclear DNA.

A second method for eliminating numts was also trialled on the problematic *Arunta* and *Thopha* individuals. This method was a very long PCR of approximately 4,000bp, from the primers trnMetF2 (C. Owen, unpublished data) to TK-N-3786 (Sueur *et al.*, 2007) in combination with the Titanium taq kit (Clontech, Mountain View, California, USA) and a cycling regime with a 45°C annealing temperature and an extension time of four minutes. The primers C1-N-2198 (Folmer *et al.*, 1994), C1-J-2195 and TL2-N-3014 were used for sequencing.

Molecular laboratory results. Genomic DNA was extracted for 19 *Thopha*, six *Arunta*, and one outgroup specimen using the Qiagen method (Table 2). COI sequence data from whole genome extractions had double peaks in several *Thopha* and two *Arunta* specimens (see Table 2). COI sequence from the mtDNA extractions had clean, single peaks in all the *Thopha* samples but not the two *Arunta interclusa* samples. COI sequence from the long PCR had clean, single peaks in the *Arunta* samples but not the *Thopha* samples. The final length of the COI sequences was 1483bp and contained no stop codons or indels (insertions or deletions). All individuals of *T. sessiliba sessiliba*, *T. s. clamoris* and *T. hutchinsoni* amplified an “intronless copy” of EF-1 α (Table 2) in addition to the functional copy of EF-1 α including introns and exons (ca. 700 bp, including 423 bp of exon). The intronless copy was not used here.

Molecular evidence for *Thopha sessiliba clamoris*. The final data alignment was done by eye. The sequences showed little alignment ambiguity, and only in intron regions of the nuclear DNA. All sequences have been deposited in Genbank with accession numbers KR108329–KR108354 (COI) and KR108355–KR108380 (EF-1 α).

The chi-square base composition test was not significant for either the COI or EF-1 α data partitions or for the combined data. Over all of the data (including the outgroup; 2229 bp), 25% of sites were variable and 22% were parsimony informative. With the outgroup excluded, 28% of COI sites, 4% of EF-1 α exon sites and 15% of EF-1 α intron sites were parsimony-informative.

The greatest sequence divergence within the tribe Thophini was between species of the two genera, *Thopha* and *Arunta*. From the COI data only, *Thopha* and *Arunta* were separated by a maximum of 0.165 uncorrected substitutions per site (corrected/patristic distances for COI only, EF-1 α only, and the combined dataset can be estimated from the trees shown in Fig. 4). COI sequence divergence between the different *Thopha* species varied from 0.122 to 0.047 uncorrected substitutions per site. Within most species the sequence divergence was large for intraspecific comparisons; *T. colorata* from the western edge of the range varied by up to 0.043 substitutions per site from individuals from the centre of the range. The smallest sequence divergences were found between the morphologically similar *T. sessiliba sessiliba*, *T. s. clamoris* and *T. hutchinsoni*. *Thopha sessiliba clamoris* differed from its sibling, *T. sessiliba sessiliba*, by 0.047 uncorrected substitutions per site, more than that observed between closely related species in other Cicadidae genera (e.g., *Kikihia*—Arensburger, 2004; Marshall *et al.*, 2011; *Maoricicada*—Buckley *et al.*, 2006; *Psithyristria*—Lee and Hill, 2010).

Molecular phylogenetic methods. The combined DNA dataset was analysed using partitioned maximum-likelihood (ML) in Garli v2.0 (Zwickl, 2006). Substitution models and partition schemes were evaluated by *PartitionFinder* (Lanfear *et al.*, 2012) under the BIC criterion from a set of six candidate data subsets incorporating different COI codon positions and/or EF-1 α introns and exons. Gamma distributions were estimated with four rate categories. Tree

topology was linked across data subsets, while evolutionary rates were allowed to vary. Default settings were used for the analysis parameters except that runs were continued without topological change for 50,000 generations rather than 20,000.

For each analysis, ten replicate Garli analyses were conducted from different starting trees to confirm repeatability of the solutions. To estimate branch support, a ML bootstrap analysis was conducted with 200 replicates, each with three heuristic search runs.

The COI and EF-1 α datasets were also analysed separately using the above methods to check for the possibility of different gene histories. Substitution models and partition schemes were separately evaluated in each case, as above.

Molecular phylogenetic results. For the combined molecular dataset, *PartitionFinder* selected five partitions (COI 1st position—K81uf+G, COI 2nd position—HKY+I, COI 3rd position—HKY, EF-1 α exons—TrNef+I, EF-1 α introns—HKY). Note that estimated base frequencies were specified for all but the exon partition. These models and partitions were confirmed for the mtDNA and nuclear-gene-only analyses.

The ML tree from the combined molecular data (Fig. 4b) matched the morphology tree (Fig. 4a) both in topology and pattern of bootstrap support. *Arunta* and *Thopha* were recovered as strongly supported monophyletic genera. Of the extant lineages within the genus *Thopha*, the one containing *T. saccata* was modelled to have diverged first, albeit with very weak support caused by conflict between the two gene subsets (see below). The two *T. sessiliba clamoris* sequences formed

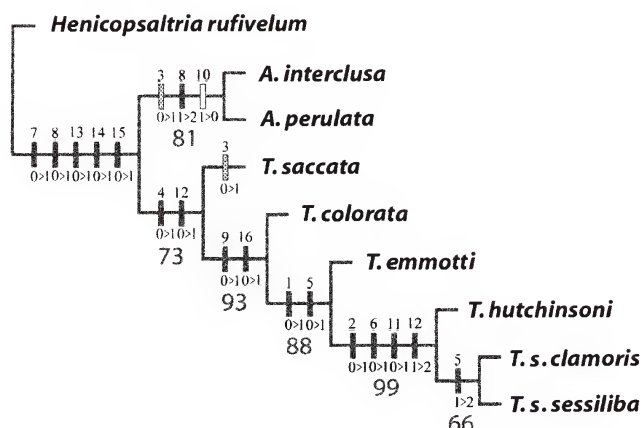
Table 2. Collecting data for specimens used in the molecular analyses. *loc.* = locality numbers for sites, see Figs. 2, 4 and 5.

taxa	loc.	specimen code	locality	latitude	longitude	date
<i>Thopha colorata</i>	3	04.AU.NT.SGT.01	NT: W of Alice Springs	23°44.088'S	133°44.068'E	29 Jan 2004
	4	04.AU.NT.SGT.02	NT: W of Alice Springs	23°44.088'S	133°44.068'E	29 Jan 2004
	5	09.AU.WA.CVN.01	WA: Carnarvon	24°53.133'S	113°39.376'E	12 Feb 2009
	6	10.AU.WA.CVN.10	WA: Carnarvon	24°53.133'S	113°39.376'E	15 Jan 2010
<i>Thopha emmotti</i>	7 ^a	08.AU.QL.BOU.01	QLD: Burke River nr Boulia	22°54.707'S	139°55.094'E	27 Feb 2008
	8 ^a	09.AU.QL.STC.01	QLD: c.30km N of Jundah	24°39.242'S	143°16.366'E	01 Feb 2009
<i>Thopha hutchinsoni</i>	9 ^{ab}	06.AU.WA.PCS.01	WA: c.300km N of Newman	21°29.889'S	118°43.935'E	11 Feb 2006
	10 ^{ab}	06.AU.WA.PCS.02	WA: c.300km N of Newman	21°29.889'S	118°43.935'E	11 Feb 2006
	11 ^{ab}	09.AU.WA.MRQ.02	WA: c.70km N of Newman	23°08.246'S	119°13.362'E	17 Feb 2009
<i>Thopha saccata</i>	12 ^a	01.AU.QL.HER.09	QLD: c.30km W of Townsville	19°21.636'S	146°27.215'E	03 Jan 2001
	13 ^a	01.AU.QL.HER.13	QLD: c.30km W of Townsville	19°21.636'S	146°27.215'E	03 Jan 2001
	14	03.AU.NS.BRI.01	NSW: Wedderburn, S of Sydney	34°09.009'S	150°49.789'E	01 Dec 2003
<i>Thopha sessiliba sessiliba</i>	15 ^b	04.AU.QL.FLN.01	QLD: Flinders River nr Richmond	20°42.996'S	143°08.382'E	06 Jan 2004
	16 ^b	05.AU.QL.TBL.01	QLD: Bloomsbury	20°41.838'S	148°35.416'E	09 Jan 2005
	17 ^b	07.AU.QL.LFS.01	QLD: 2.7km S of Lakefield	14°56.989'S	144°12.523'E	11 Jan 2007
	18 ^{ab}	08.AU.QL.BOS.01	QLD: Burke River S of Boulia	22°55.560'S	139°55.231'E	28 Feb 2008
	19 ^{ab}	06.AU.WA.OUT.03	WA: 37km E of Fitzroy Crossing	18°27.532'S	125°45.172'E	06 Feb 2006
<i>Thopha sessiliba clamoris</i>	1 ^{ab}	09.AU.WA.MRP.02	WA: c.78km N of Newman	23°07.178'S	119°06.332'E	17 Feb 2009
	2 ^{ab}	09.AU.WA.GRS.01	WA: c.146km N of Meekatharra	25°35.579'S	119°14.173'E	18 Feb 2009
<i>Arunta interclusa</i>	20 ^a	08.AU.QL.MKY.01	QLD: Near Mackay	21°08.631'S	149°10.232'E	02 Mar 2008
	21 ^a	09.AU.QL.NSP.03	QLD: Surfers Paradise	27°59.477'S	153°25.718'E	05 Jan 2009
<i>Arunta perulata</i>	22	07.AU.QL.RSN.01	QLD: Rollingstone	19°02.70'S	146°23.75'E	05 Jan 2007
	23	08.AU.QL.ALM.01	QLD: NE of Ayr nr Alva Beach	19°28.014'S	147°28.321'E	21 Dec 2008
	24	09.AU.QL.SSN.01	QLD: Southport Spit	27°56.339'S	153°25.524'E	05 Jan 2009
	25	97.AU.NS.CWD.74	NSW: Crowdy Bay	31°45'S	152°45'E	14 Feb 1997
<i>Henicopsaltria rufivelum</i>		04.AU.QL.BFA.01	QLD: Near Kuranda	16°49.498'S	145°37.876'E	09 Jan 2004

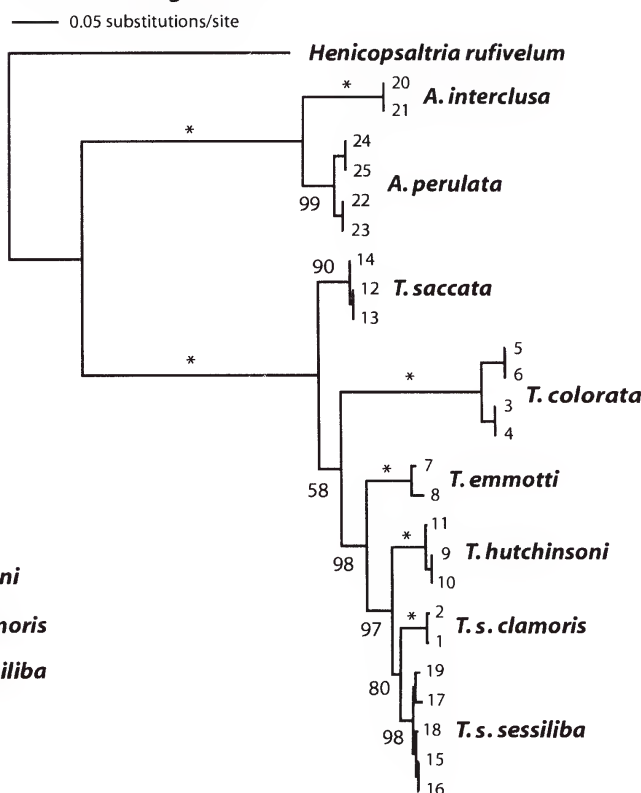
^a COI numt co-amplified when PCR performed on genomic DNA

^b intronless copy of EF-1 α present

a) Morphological characters



b) Combined genetic characters



c) mtDNA characters (COI)

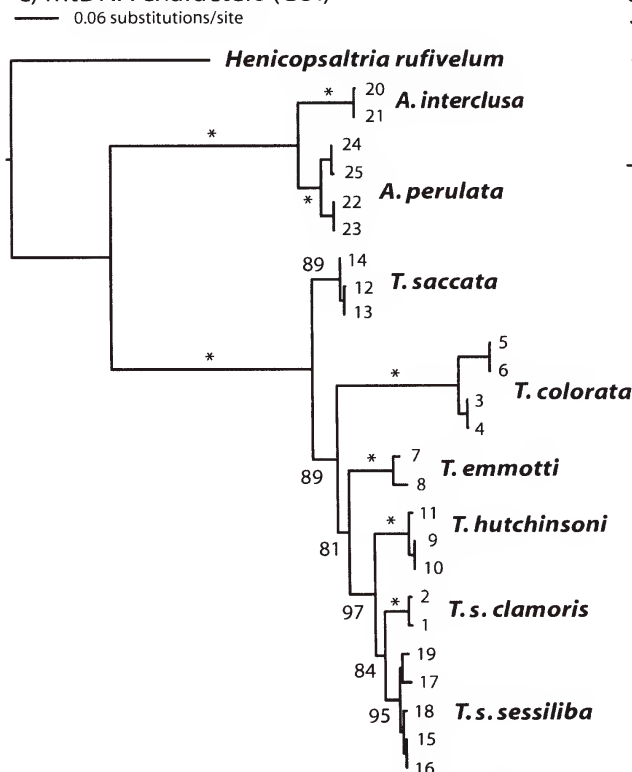
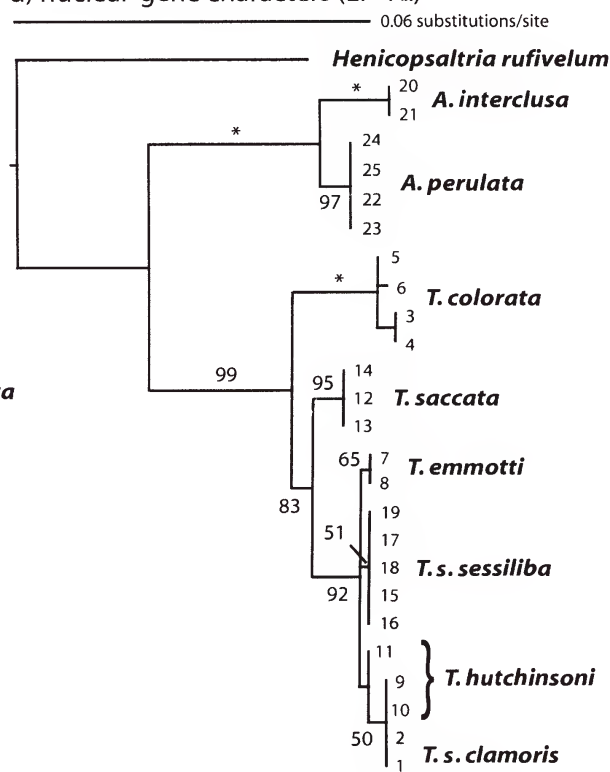
d) nuclear-gene characters (EF-1 α)

Figure 4. Phylogenetic relationships of the Thophini (viz. *Thopha* and *Arunta* species), with respect to the outgroup *Henicopsaltria rufivelum*. Tip numbers correspond to locations in Table 2. (a) tree from parsimony analysis of morphological characters; (b–d) trees from maximum-likelihood analyses of the combined genetic data, the mtDNA COI data alone, and the nuclear-gene data alone. Numbers at nodes are bootstrap percentages from 1000 replicates (morphology) and 200 replicates (molecular). Morphological character transformations are shown as: black bars = non-homoplasious forward change; grey bars = homoplasious forward change; white bar = reversal (whether homoplasious or not); * = 100%. InL scores of the molecular trees are as follows: combined InL = 7331.59, COI = 5676.00, EF-1 α = 1605.37.

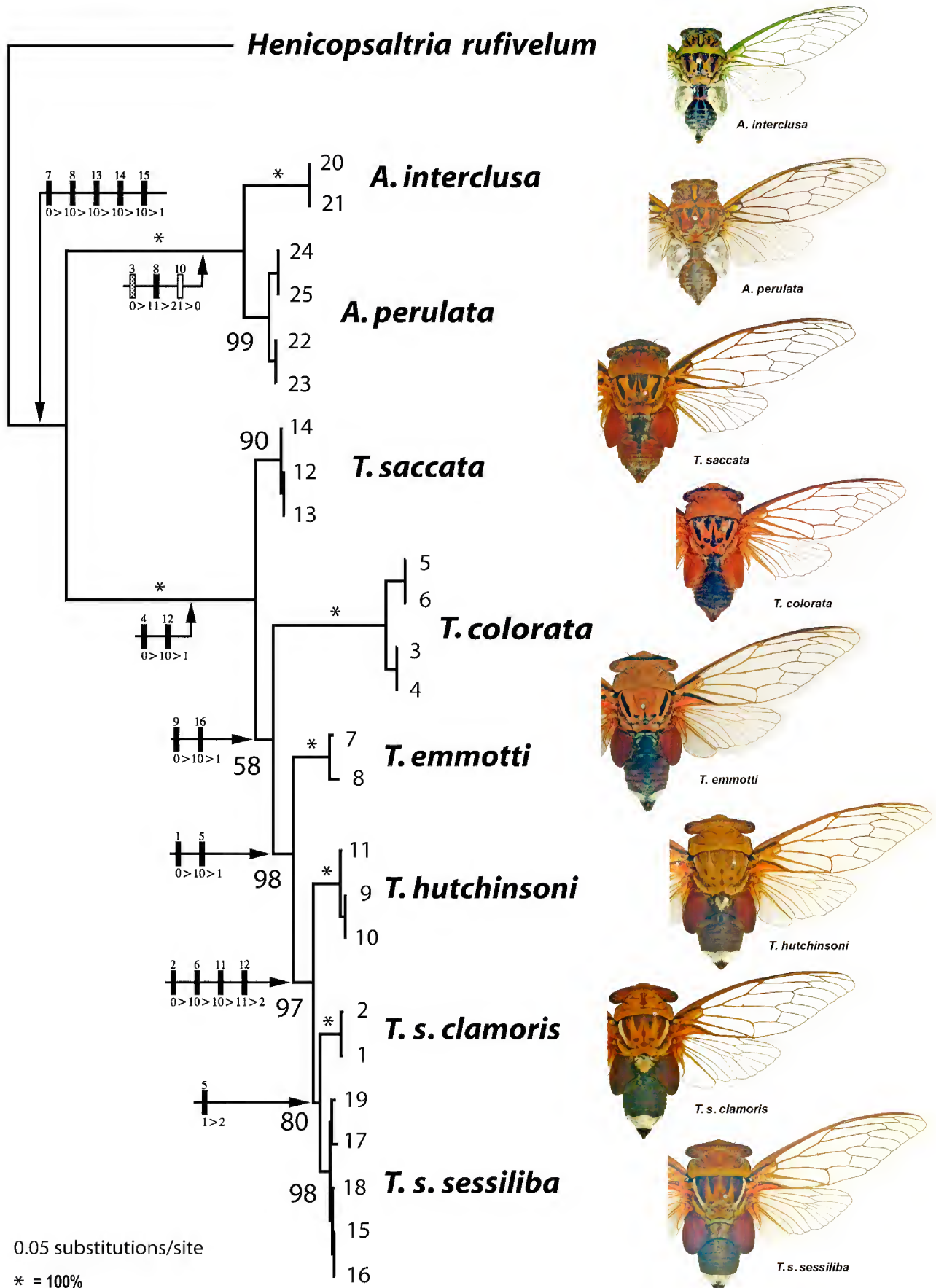


Figure 5. Thophini phylogeny of combined molecular data superimposed with morphological transformations (the two trees being identical in topology) together with habitus images of all seven Thophini species. See Fig. 4 for explanation of tree data.

a well-supported clade sister to *T. sessiliba sessiliba*, with *T. hutchinsoni* as the next closest relative (also well-supported).

The COI-only and EF-1 α -only trees (Fig. 4c and 4d) broadly matched the combined-data tree, with two notable instances of conflict. First, COI strongly supported the initial split of *Thopha saccata* or its ancestor from its congeners, while EF-1 α recovered *T. colorata* as the first split, again with reasonable support (83% bootstrap), followed by *T. saccata*. Second, while the four remaining *Thopha* species were clearly distinguishable and well-supported in the COI tree, with *T. sessiliba clamoris* sister to *T. s. sessiliba*, the *T. s. clamoris* sequences were interdigitated with those of *T. hutchinsoni* in the EF-1 α tree, and there was only very weak signal for distinguishable *T. emmotti* and *T. sessiliba* species.

Acoustic analysis of *Thopha* songs

The songs of all *Thopha* species are being investigated by A. Ewart (pers. comm.). For this paper, following our phylogenetic results above, we restricted our analysis to the question of whether the songs of *T. sessiliba sessiliba*, *T. sessiliba clamoris* and *T. hutchinsoni* are distinguishable from each other.

Song analysis methods. Field recordings were made using Marantz PMD660, PMD670, or PMD680 digital audio recorders (Marantz America Inc, Mahwah, New Jersey, USA), connected to either a Sennheiser omnidirectional ME62 microphone (Sennheiser Electronic Corp USA, Old Lyme, Connecticut, USA) mounted inside a Sony PBR-330 parabola (Sony Electronics Inc, Fort Myers, Florida, USA) or a Sennheiser ME66 short shotgun microphone without parabola. Microphones were powered with a Sennheiser K6 power module. Recordings were sampled at 44–48 kHz. Recorded songs were analysed using Raven Pro v1.4 (Cornell Lab of Ornithology, Ithaca, New York, USA).

One recording per specimen was used for analysis. For each recording, ten samples were taken from segments dominated by the song of one male, with each sample corresponding to an interval containing 30 pulses (presumed to be made by individual or synchronous opposing timbal clicks) (see Fig. 1d). In a few recordings of *T. sessiliba sessiliba*, 15 pulse segments were used because of poor recording quality and/or fewer than ten samples were taken. Each sample was used to estimate the primary pulse rate (pulses per second) and the dominant frequency of the song. The ten samples were averaged for each individual, and the mean values for the individuals of each species were averaged. Three recordings from one location were available for *Thopha sessiliba clamoris*, 13 recordings from 13 locations for *T. s. sessiliba* and 10 recordings from five locations for *T. hutchinsoni* (Table 3).

Song analysis results. *Description of Thopha sessiliba clamoris* song. The song of *T. s. clamoris*, like those of the other *Thopha* species except *T. colorata*, is an extremely loud whine, much like an institutional fire alarm, that varies in amplitude and contains complex oscillating harmonics that are difficult to measure and quantify (see Fig. 1d). The sound energy forms a single roughly symmetrical peak, dropping 20 dB at 3.7 and 6.4 kHz from its dominant frequency of approximately 5 kHz. The waveform in *T. s. clamoris* oscillates between series of pulses of approximately 2 ms duration (here presumed to be timbal-clicks), produced

at about 240/sec, and series of weaker-amplitude paired pulses in which the pairs themselves appear at about 240/sec, suggesting left and right timbals moving in and out of phase. In paired pulse sections, the leading or lagging pulse was often stronger. The shift between single and paired clicks varies in timing but occurs approximately every 100 to 300 ms. Individual males also switch occasionally between continuous song and pulsed song. The overall acoustic impression is either a fairly smooth “deeuueeuueeuueeu” or a pulsed “deeu deeu deeu deeu deeu”, with an individual continuing for seconds to minutes in one mode or the other.

Detailed song measurement. Plots of dominant frequency versus timbal click rate (major pulses) showed clear differentiation for *Thopha hutchinsoni*, but only a suggestion of differentiation for *T. sessiliba clamoris* and *T. sessiliba sessiliba*. The means and ranges (in pulses per second) for the pulse rate character for *T. s. clamoris*, *T. s. sessiliba* and *T. hutchinsoni* were as follows, respectively: 239 (231–244), 196 (187–206), and 263 (220–312). The means for dominant frequency (in kHz), in the same order, were as follows: 5.00 (4.86–5.11), 4.67 (4.52–4.86, and 5.11 (4.88–5.44). A scatterplot of the data (Fig. 3) showed clear separation of the *T. hutchinsoni* songs, with the one recording from the geographically intermediate site WA.KRH falling between the *T. hutchinsoni* cluster and that of *T. s. clamoris* + *T. s. sessiliba*. The latter two taxa were not distinguished, although the *T. s. clamoris* points clustered on one side of the range observed within *T. s. sessiliba*. There was no clear geographic pattern within the *T. s. sessiliba* data, with both eastern and western samples overlapping the *T. s. clamoris* data points.

Discussion

Molecular and morphological relationships. The topography of the single tree derived from the morphological cladistic analysis agrees exactly with the trees based on molecular data for mtDNA characters (COI). The molecular tree derived from nuclear-gene characters (EF-1 α) differed only in the transposed positions of *T. saccata* and *T. colorata*. The combined molecular-data tree was identical to the cladistic tree and mtDNA tree.

The only morphological differences between *T. sessiliba clamoris* and its sister *T. sessiliba sessiliba* were differences in colour markings (although these were striking). However, the mtDNA (COI) tree supports the differentiation between the two, as it does for all other recognized *Thopha* species, albeit to a lesser degree (0.06 corrected substitutions per site) than between other pairs of congeners (0.12 between *T. s. sessiliba* and *T. hutchinsoni*) to a maximum of 0.26 between *T. saccata* and *T. colorata*. The differentiation of the *T. s. clamoris* and *T. s. sessiliba* clades is further supported by strong bootstraps, the clade of *T. s. clamoris* (2 specimens) by a bootstrap of 100% and the clade of *T. s. sessiliba* (5 specimens) by 95% in the COI tree and 98% and 100% respectively in the combined analysis.

It is interesting to note that the two populations of *T. colorata*, two specimens from central Australia and two from Carnarvon in the far west of the continent over 1,600 km away, show a genetic difference not unlike the two subspecies of *sessiliba* and somewhat similar to those between some of the most closely related species of *Thopha*. However, like the east and west populations of *sessiliba* we do not consider these populations separate species (or subspecies)

Table 3. *Thopha* specimens used in the song analysis. The number of recordings for each site is followed by the site code and the locality data; *n* = number of specimens.

taxa	<i>n</i>	location code	locality	latitude	longitude	date
<i>Thopha hutchinsoni</i>	1	AU.WA.MRS	WA: c.112km NW of Newman	23°03.386'S	118°51.977'E	12 Feb 2006
	1	AU.WA.PCS	WA: c.300km N of Newman	21°29.889'S	118°43.935'E	11 Feb 2006
	4	AU.WA.MRQ	WA: c.70km N of Newman	23°08.246'S	119°13.362'E	17 Feb 2009
	3	AU.WA.NEN	WA: 34km N of Newman	23°13.758'S	119°29.423'E	17 Feb 2009
	1	AU.WA.TOP	WA: Nanutarra-Wittenoom Rd	22°22.954'S	117°53.140'E	15 Feb 2009
<i>Thopha sessiliba clamoris</i>	3	AU.WA.GRS	WA: c.146km N of Meekatharra	25°35.579'S	119°14.173'E	18 Feb 2009
<i>Thopha sessiliba sessiliba</i>	1	AU.QL.MLR	QLD: near Mt. Carbine	16°33.574'S	145°05.054'E	10 Jan 2004
	1	AU.QL.HMT	QLD: Herberton Mine	17°23.173'S	145°22.674'E	09 Jan 2004
	1	AU.QL.TBL	QLD: Bloomsbury	20°41.838'S	148°35.416'E	09 Jan 2005
	1	AU.QL.LFS	QLD: 2.7km S of Lakefield	14°56.989'S	144°12.523'E	11 Jan 2007
	1	AU.QL.QRR	QLD: c.63km SW of Bowen	20°23.140'S	147°53.133'E	09 Jan 2009
	1	AU.QL.GRD	QLD: c.50km S of Cape River	21°19.101'S	146°31.352'E	21 Jan 2005
	1	AU.QL.FLN	QLD: Flinders River nr Richmond	20°42.996'S	143°08.382'E	06 Jan 2004
	1	AU.QL.BOS	QLD: Burke River S of Boulia	22°55.560'S	139°55.231'E	28 Feb 2008
	1	AU.QL.URD	QLD: Moonah Ck, S of Mt. Isa	21°22.943'S	139°02.672'E	07 Feb 2010
	1	AU.NT.RTG	NT: 13 km S of Three Ways	19°33.584'S	134°13.405'E	27 Jan 2004
	1	AU.WA.OUT	WA: 37km E of Fitzroy Crossing	18°27.532'S	125°45.172'E	06 Feb 2006
	1	AU.WA.MIN	WA: Fitzroy River, S of Derby	17°44.604'S	123°35.306'E	19 Jan 2010
	1	AU.WA.DBE	WA: Derby	17°18.973'S	123°39.115'E	19 Jan 2010
	1	AU.WA.KRH	WA: Kumarina Roadhouse	24°42.590'S	119°36.510'E	17 Feb 2009
unknown (not collected)	1	AU.WA.KRH	WA: Kumarina Roadhouse	24°42.590'S	119°36.510'E	17 Feb 2009

because there are no morphological or colour differences between them. The genetic difference is most likely due to extermination of intermediate populations caused by environmental changes related to progressive aridification commencing in and continuing through the mid to late Pliocene and continuing into the Pleistocene.

Songs. The songs of all *Thopha* species are remarkably similar when compared to differences between species in many other genera, especially given the substantial DNA divergence within the group. Our few song samples do not distinguish *T. sessiliba clamoris* and *T. sessiliba sessiliba*, and this is a major reason for the decision to name *T. s. clamoris* at the subspecies level. However, there are sufficient differences in song between *clamoris* + *sessiliba* and their sister *T. hutchinsoni* to confirm *hutchinsoni* as distinct from *sessiliba* and *clamoris*. This is fortuitous as it confirms the species distinction made between *clamoris* + *sessiliba* and *T. hutchinsoni* in the morphological and CO1 trees despite the confused (and weakly supported) placement of *T. hutchinsoni* in the EF-1 α tree where it merges with *T. s. clamoris*. Regardless, *T. hutchinsoni* is sufficiently distinct in its appearance not to be mistaken for either *T. s. sessiliba* or *T. s. clamoris*.

Distribution

There is a correlation between the phylogenetic position of *Thopha* species and their geographic distributions. *T. saccata*, the sister to all other *Thopha* species in the combined-gene tree and morphological tree, is distributed along the eastern fringe of the continent and is the only species whose distribution reaches a temperate climate. This pattern is intuitively appealing because the sister-genus *Arunta* is coastal in distribution, although since the EF-1 α tree places *T. colorata* as sister to the remaining *Thopha*

species this question remains open for future study. *Thopha colorata*, the next-branching species in the combined-gene tree and morphological tree, is an enigma occurring in arid parts of central Australia and in the arid far west of the continent. However, the next-branching species, *T. emmotti* and *T. hutchinsoni*, are also arid species with their distributions in similar latitudes to the populations of *T. colorata*. *Thopha sessiliba sessiliba* and *T. s. clamoris* represent the most recently diverged taxa; together they are sister to *T. hutchinsoni*. *Thopha s. sessiliba* has a very wide distribution across the monsoonal north of Australia with the limits of its distribution penetrating the arid centre and becoming virtually sympatric with the distributions of the arid-loving *T. colorata* and *T. emmotti*. *Thopha s. clamoris* appears to be a western extension of the distribution of *T. s. sessiliba* that has become isolated by drying of the Great Sandy Desert in the north-west of the continent, one of the few regions in Australia where there are no *Eucalyptus* at all.

New distribution records for *Thopha* species

***Thopha emmotti* Moulds, 2001.** Queensland: 1 male (molecular voucher AU.QL.BOU.01) Burke R. xing, SE of Boulia on Kennedy Dev. Rd, 22°54.707'S 139°55.094'E, 27 Feb. 2008, Hill, Marshall, Moulds, Owen & Humphrey (MSM).

***Thopha sessiliba sessiliba* Distant, 1892.** Western Australia: 1 male, nr Nicholson R., 145 km E of Halls Creek, 18°08.41'S 12°842.003'E, 23 Jan. 2010, K. Hill & D. Marshall (MSM). QUEENSLAND: 1 male (molecular voucher AU.QL.BOS.01), Burke R. xing, SE of Boulia on Kennedy Dev. Rd, 22°54.707'S 139°55.094'E, 27 Feb. 2008, Hill, Marshall, Moulds, Owen & Humphrey; 3 males, 1 female, Noonbah Stn, 24°07'S 143°11'E, 4 Feb. 2004, A.J. Emmott; 7 males, 3 females, Spring Pond, Noonbah Stn, SW of Longreach,

24°04'S 143°11'E, A.J. Emmott & P. Kleinschmidt; 2 males, 4 females, 42 km N of Wyandra, 26°53.616'S 146°03.428'E, 4 Jan. 2005, Hill, Marshall, Moulds (MSM).

These records extend the known distribution of this subspecies a little to the south-east in Western Australia, and considerably further west in Queensland to a point where it overlaps the distribution of *T. emmotti* near Boulia and at Noonbah Station.

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